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This project investigates the symbiosis between anomalopids (flashlight fishes) and their luminous bacteria symbionts. Anomalopid fishes harbor luminous bacterial symbionts in large light organs located beneath the eyes. This association is one of the most highly evolved extracellular bacterial symbioses, and the symbionts have resisted attempts to culture them. This project focuses on two major aspects of the symbiosis, 1) evolution and 2) establishment of the symbiosis in anomalopid larvae. During the current period we have done genetic comparisons of symbionts and culturable luminous bacteria. We find this symbiosis appears to be quite different from other light organ symbioses in that the symbionts are closely tied to the hosts and appear to have diverged along with them.

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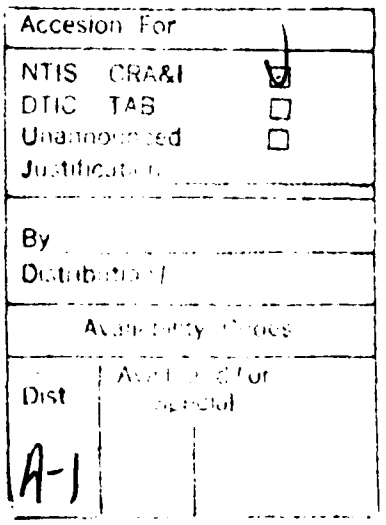

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ANNUAL REPORT
ONR GRANT N00014-89-J-1742

Principal Investigator: Margo G. Haygood

Grantee: University of California, San Diego
Scripps Institution of Oceanography

Grant Title: Flashlight Fish Symbiosis

Start Date: 1 March 1989

Project Objective:

1. To investigate the evolution of the symbiosis between luminous bacteria and anomalopid fishes. Specific questions to be addressed:

    a. To determine if the *Kryptophanaron alfredi* symbiont belongs to any of the known free living bacteria, and if not what are its closest free living relatives?

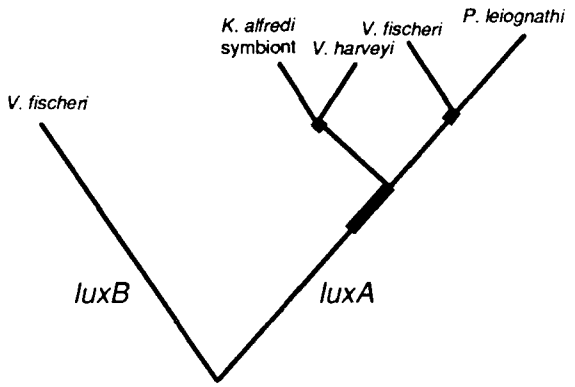
    b. To ascertain whether the symbionts from this family of fishes are all members of one species of bacteria, as is the case in all other light organ symbioses (with culturable symbionts) that have been studied, or alternatively,whether the symbionts have diverged with the hosts.

2. To test the feasibility of investigating the initiation of the symbiosis in flashlight fish larvae.

Progress (Year 1):

Flashlight fishes (family Anomalopidae) have light organs that contain luminous bacterial symbionts. Although the symbionts have not yet been successfully cultured, the luciferase genes have been cloned directly from the light organ of the Caribbean species, *Kryptophanaron alfredi.*

In order to address objective 1a above, a comparison of *lux* genes among the *Kryptophanaron alfredi* symbiont and culturable luminous bacteria was undertaken. Hybridization of a *lux* probe consisting of *luxA* and about half of *luxB* from the *Kryptophanaron alfredi* symbiont to DNAs from 9 strains (8 species) of luminous bacteria showed that none of the strains tested had *lux* genes highly similar to the symbiont. The most similar were a group consisting of *Vibrio harveyi*, *Vibrio splendidus* and *Vibrio orientalis*. The nucleotide sequence of the luciferase α subunit gene (*luxA*) of the *Kryptophanaron alfredi* symbiont was determined in order to do a more detailed comparison with published *luxA* sequences from *Vibrio harveyi*, *Vibrio fischeri* and *Photobacterium leiognathi*. The hybridization results, sequence comparisons and the mol% G+C of the *Kryptophanaron alfredi* symbiont *luxA* gene suggest that the symbiont is a new species of luminous *Vibrio* related to *Vibrio harveyi*. A phylogenetic tree based on analysis of *luxA* and *luxB* amino acid sequences with the PHYLIP protein parsimony algorithm version 3.2 is shown below.



Divergence of *lux* genes. Branch lengths are proportional to amino acid replacements. Heavy bars represent uncertainty in branch length (minimum to maximum replacements).

The outgroup was *luxB* since it is believed to be the result of gene duplication of *luxA*. The results of the phylogenetic analysis are consistent with enzyme kinetics and hybridization data. Since the *K. alfredi* symbiont and *V. harveyi* genes have diverged almost as much as those of *V. fischeri* and *P. leiognathi*, which are distinct species currently assigned to different genera (although both were classified as *Photobacterium* in the past), that degree of difference suggests that the symbiont is probably not *V. harveyi*, but a different species of *Vibrio*. This work has been submitted for publication.

In order to investigate objective 1b above, an extensive RFLP analysis of anomalopid symbionts from three genera and four species, and two strains each of three species of culturable luminous bacteria was undertaken. Both *lux* and 16S rRNA probes were used. A manuscript based on this project is in preparation. The primary conclusions are:

1. The relationships among the symbionts appear to correspond to the proposed host phylogeny (i.e. *Anomalops katoptron* symbionts are most divergent), supportive of co-evolution.

2. Symbionts from hosts of two different genera collected in the same location are as different from each other as they are from symbionts from a third genus from another location. This implies that the symbionts are tied to the hosts, and are not derived from a common free-living population.

3. The symbionts from different host genera differ greatly from each other. The divergence is comparable to that between species of culturable luminous bacteria. Although the symbionts are clearly derived from a common ancestor, and are more related to each other than to the other bacteria tested, this degree of divergence implies that unlike the other light organ symbioses, members of the family Anomalopidae have symbionts that belong to different species.

4. Puzzling results were obtained with the *Photoblepharon* samples. Divergence among these samples ranged from a level comparable to strain differences among culturable bacteria, to species level divergence. We clearly need to obtain a larger number of samples from this genus.

Work Plan:

1. We plan to obtain *lux* and 16S rRNA sequence data from symbionts to provide more precise estimates of divergence. We will also attempt comparisons of more samples from whatever host species we can obtain.
2. We will participate in a cruise Sept 1990 to collect angler fish samples for analyses similar to those above.
3. To address objective 2 we will do field work in May-June 1990 in Roatan Honduras to attempt to raise *K. alfredi* larvae and examine light organ development.

Inventions:

None

Publications:

Haygood, M.G. Relationship of the luminous bacterial symbiont of the Caribbean flashlight fish, *Kryptophanaron alfredi* (family Anomalopidae) to other luminous bacteria based on bacterial luciferase (*lux*) genes. submitted to Arch. Microbiol.

Wolfe, C.J. and M.G. Haygood. Genetic divergence among anomalopid symbionts and free living luminous bacteria determined by RFLP analysis with *lux* and 16S rRNA gene probes. in preparation.

Training Activities:

One graduate student, Connie Wolfe (female) is supported on this project. One undergraduate student, Arlyne Beeche (female, minority) also worked on this project.

Awards/Fellowships:

None